



# Inter- and intra-specimen variability masks reliable temperature control on shell Mg/Ca ratios in laboratory and field cultured *Mytilus edulis* and *Pecten maximus* (bivalvia)

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# Inter- and intra-specimen variability masks reliable temperature control on shell Mg/Ca ratios in laboratory and field cultured *Mytilus edulis* and *Pecten maximus* (bivalvia)

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## Abstract

The Mg/Ca ratios of biogenic calcites are commonly seen as a valuable palaeo-proxy for reconstructing past ocean temperatures. The temperature dependence of Mg/Ca ratios in bivalve calcite has been the subject of contradictory observations. The palaeoceanographic use of a geochemical proxy, like Mg/Ca ratios, is dependent on initial, rigorous calibration and validation of relationships between the proxy and the ambient environmental variable to be reconstructed. Shell Mg/Ca ratio data are reported for the calcite of two bivalve species, *Mytilus edulis* (common mussel) and *Pecten maximus* (king scallop), for the first time grown in laboratory culturing experiments at controlled and constant aquarium seawater temperatures over a range from ~10 to ~20°C. Furthermore, Mg/Ca ratio data of laboratory-grown and field-grown *M. edulis* specimens were compared. Only a weak, albeit significant, shell Mg/Ca ratio–temperature relationship was observed in the two bivalve species: *M. edulis* ( $r^2=0.37$ ,  $p<0.001$  laboratory cultured specimens and  $r^2=0.50$ ,  $p<0.001$  for field cultured specimens) and *P. maximus* ( $r^2=0.21$ ,  $p<0.001$ , laboratory cultured specimens only). In the two species, shell Mg/Ca ratios also were not found to be controlled by shell growth rate and salinity. Furthermore, measurement of Mg/Ca ratios in the shells of multiple specimens illustrated that a large degree of variability in the measured shell Mg/Ca ratios was significant at the species, inter- and intra-individual shell levels. The study data suggest the use of bivalve calcite Mg/Ca ratios as a reliable, precise and accurate temperature proxy still remains limited, at least in the species studied to date. Such limitations are most likely due to the presence of significant physiological effects on Mg incorporation in bivalve calcite, with such variability differing both within single shells and between shells of the same species that were precipitated under the same ambient conditions.

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## 1 Introduction

Carbonate skeletal remains, i.e. foraminifera, corals, ostracodes and bivalves, are valuable archives of information for palaeo-reconstruction of changes in physical and chemical oceanographic conditions. The incremental growth of biogenic carbonates, such as the shells of marine bivalve molluscs or the coral skeleton, has the potential to record high-resolution time-series of those environmental conditions in which the organism grew. Furthermore, marine bivalves occupy widely distributed habitats in the modern-day oceans, as well as being relatively common throughout the fossil record since the Cretaceous. Information on past environmental conditions that are preserved in carbonates can be obtained through the use of proxies, i.e. physical and chemical signals that provide information on sought after variables that cannot be measured directly, such as seawater temperature or salinity. However, a proxy is rarely dependent on a single variable, and the influence of other secondary independent variables complicates, to a lesser or greater extent, proxy use in palaeo-studies; such factors must be assessed rigorously via calibration and validation studies prior to successful application (for reviews, see e.g. Wefer et al., 1999; Lea, 2003).

The use of the oxygen-isotope composition ( $^{18}\text{O}/^{16}\text{O}$  ratios expressed as  $\delta^{18}\text{O}$  values) of biogenic carbonate archives as a proxy for seawater temperature (for reviews, see e.g. Emiliani, 1966; Wefer and Berger, 1991) is one of the most powerful tools in palaeoceanographic studies (e.g. Shackleton, 1967; Shackleton and Opdyke, 1973; Gagan et al., 2000), but its use is complicated by factors other than temperature, namely variation in the oxygen-isotope composition of seawater, pH and kinetic effects (e.g. McConnaughey, 1989; Spero et al., 1997). By comparison, the predicted thermodynamic control of  $\text{Ca}^{2+}$  substitution by  $\text{Mg}^{2+}$  in inorganically precipitated calcite (Chilingar, 1962; Katz, 1973; Mucci, 1987; Oomori et al., 1987) and the observed temperature dependence of Mg/Ca ratios in some biogenic calcites (Chave, 1954; Dwyer et al., 1995; Klein et al., 1996; Nurnberg et al., 1996; Rosenthal et al., 1997; Lea et al., 1999; Dwyer et al., 2000; Elderfield and Ganssen, 2000; Lear et al., 2002)

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have resulted in Mg/Ca ratios being seen as a salinity-independent temperature proxy that makes an ideal companion to the  $\delta^{18}\text{O}$ -temperature proxy. However, the clear species-specific temperature dependence of Mg/Ca ratios that has been observed in foraminiferal calcite (Rosenthal et al., 1997; Lea et al., 1999; Elderfield and Ganssen, 2000; Lear et al., 2002) suggests that parameters other than temperature also can influence the Mg/Ca ratios of biogenic calcites. For example, biological influences such as gametogenesis, ontogeny, growth rate and size, as well as environmental and physical parameters such as salinity, pH and post-depositional dissolution, have all been proposed to significantly influence foraminiferal Mg/Ca ratios (Delaney et al., 1985; Lea et al., 1999; Elderfield et al., 2001; Bentov and Erez, 2005). Furthermore, observations of significant small-scale intra-shell heterogeneity in Mg contents indicates a strong physiological control on the Mg/Ca ratio of biogenic calcites, such as observed in foraminifera (Rio et al., 1997; Hathorne et al., 2003; Eggins et al., 2004; Bentov and Erez, 2005; Sadekov et al., 2005), ostracodes (Rio et al., 1997) and bivalves (Lorens and Bender, 1980; Rosenberg et al., 2001).

In calcitic bivalve molluscs the occurrence of a temperature control on shell Mg/Ca ratios has been the subject of several studies that have returned contrasting results, but nevertheless shell Mg/Ca ratios have been used to reconstruct palaeotemperatures from fossil bivalves (Klein et al., 1997; Immenhauser et al., 2005). In an early study, a weak positive correlation between shell calcite Mg concentration with temperature was reported for three species from the genus *Mytilus* (Dodd, 1965). More recently, Klein et al. (1996) described a clear temperature dependence of Mg/Ca ratios for the mussel *Mytilus trossulus*, a close relative of *Mytilus edulis* that some consider to be a sub-species in the *M. edulis* species complex (Gardner, 1992; Riginos and Cunningham, 2005). Vander Putten et al. (2000) observed a similar relationship for *M. edulis* (blue mussel), but with an apparently seasonal breakdown in the relationship between Mg/Ca and temperature also being reported. A clear seasonal relationship between shell Mg/Ca ratios and calcification temperature for the large fan mussel *Pinna nobilis* has also been reported, albeit with an additional ontogenetic influence

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(Freitas et al., 2005). For other bivalve species, such as *Pecten maximus* (king scallop), there also exists no clear temperature relationship; Lorrain et al. (2005) reported an absence of a significant correlation between Mg/Ca ratios and temperature for this species while a weak, albeit significant, Mg/Ca ratio to temperature relationship was also observed (Freitas et al., 2006), with the relationship breaking down during winter months. Furthermore, several studies report, or suggest, the occurrence of significant non-thermodynamic controls on the Mg content of bivalve mollusc calcite, such as salinity (Dodd, 1965), solution Mg/Ca ratios (Lorens and Bender, 1980) or the animal's metabolism (Lorens and Bender, 1977, 1980; Vander Putten et al., 2000). Significant small-scale heterogeneity in Mg content also has been described for bivalve shell calcite. Such variability has been associated with stress (Lorens and Bender, 1980), metabolic activity (Rosenberg and Hughes, 1991) and control of shell crystal elongation (Rosenberg et al., 2001).

The purpose of this study was to advance an understanding of the degree of variability of Mg/Ca ratios in calcite bivalve shells using a controlled laboratory aquarium culturing approach. Specifically, no laboratory calibration of the Mg/Ca ratio–temperature relationship in bivalve calcite has previously been performed under constrained and constant seawater temperatures. This approach is a significant advancement on previous studies, since it enables manipulation of specimens, control of environmental variables, and measurement of other parameters, such as size and growth rate. It must be acknowledged, however, that laboratory aquaria are not a true representation of the animal's natural habitat. However, the outcomes of laboratory culturing studies are only of value when validated by field-based studies, albeit with the latter suffering from a lesser degree of constraint of environmental variables. In summary, the ultimate goal of this investigation was to determine whether a reliable calibration of the Mg/Ca ratio–temperature relationship could be obtained for the shell calcite from two bivalve species, *Mytilus edulis* (blue mussel) and *Pecten maximus* (king scallop), grown under constrained and constant temperature laboratory aquaria conditions. Finally, the *M. edulis* laboratory culturing data have been compared with data from field-grown

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specimens of this same species.

## 2 Material and methods

### 2.1 Laboratory culture experiment

Two species of marine bivalve mollusc were cultured in constant-temperature aquaria in the School of Ocean Sciences, University of Wales Bangor, UK *Mytilus edulis* specimens were collected in December 2003, from naturally settled spat (1 cm<size<2 cm; age<one year) in Cable Bay, a site on the coast of Anglesey, northwest Wales, while *Pecten maximus* specimens (1 cm<size<2 cm; age<one year) were collected from a commercial fishery, Ramsay Sound Shellfish, Isle of Skye, Scotland, in November, 2003. Once moved into the laboratory environment, all animals were acclimated at a temperature of ~13°C for more than two months. Subsequently, animals of similar size were moved into separate aquaria each under different but constant temperatures and controlled food and light conditions; the aquaria were routinely cleaned of all detritus. For the duration of the experiments, animals were kept in individual plastic mesh cages within each aquarium. Acclimation to the different temperatures in each aquarium was achieved by increasing/decreasing water temperature by 1°C every 2 d before commencement of the experimental periods. A mixed algae solution of *Pavlova lutheri*, *Rhinomonas reticulata* and *Tetraselmis chui* was collected every morning from stock cultures, split into equal volumes of eight litres and then supplied to the aquaria, from containers with a drip-tap, throughout that day at rates of ~5.5 ml/min. Because of the limited number of aquaria available, two separate temperature-controlled experiments were completed with three aquaria used in each.

The two separate culturing experiments were performed to evaluate the influence of temperature on shell Mg/Ca ratios of *M. edulis* and *P. maximus* in the laboratory environment. During experiment one, from 23rd February to 7th April 2004, nominal seawater temperatures in the three available aquaria were maintained at 12, 15 and

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18°C, and only *M. edulis* was cultured. In experiment two, from 6th May to 18th June 2004, nominal seawater temperatures were maintained at 10, 15 and 20°C and both *M. edulis* and *P. maximus* were cultured. In each aquarium, with individual thermostat temperature control via a heating/glycol cooling system, the nominal seawater temperatures were controlled by setting upper and lower temperature thresholds on the individual control systems, with a maximum resolution achievable by these controllers of 1°C. For improved constraint, seawater temperature also was monitored in each aquarium every 15 min using submerged temperature loggers (Gemini Data Loggers TinyTag – TGI 3080; accuracy of  $\pm 0.2^\circ\text{C}$ ). The intermittent lack of temperature control in some aquaria is a limitation of the aquarium system used and most manifest at the lowest nominal temperature of 10°C, when the cooling system sometimes struggled to compensate for fluctuations in the temperature of the external seawater supply. Natural seawater is pumped from the proximal Menai Strait into settling tanks before being introduced as a common supply into the laboratory aquaria.

Once the animals had acclimatised, individual specimens were removed at weekly intervals (with the exception of the last growth interval in experiment two, which was longer than a week for both the 15°C and 20°C aquaria) to be processed. Each time the *M. edulis* specimens were removed from the aquaria they were exposed to the air for 5 to 6 h, while *P. maximus* specimens were kept in small holding tanks for periods of 30 to 45 min. Both methods resulted in emplacement of a disturbance mark on the surface of the shells. The shells then were photographed and digitally imaged using the AnalySIS software package. The combination of disturbance marks and photographs was used to identify and measure all shell growth between emersions and provided a time control of the new shell growth laid down throughout the experiments. Subsequently the term “growth interval” has been used to describe the time intervals between emersions of animals (Table 1). The duration of the experiments, and hence the number of growth intervals, varied with species and aquarium temperature (Table 1).

Seawater samples for measurement of salinity and  $\delta^{18}\text{O}_{\text{seawater}}$  were collected using sealed Winchester glass bottles every other day from the 15°C aquarium in both

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experiments. Samples were collected from one aquarium only, since the seawater supply was common in all aquaria and water turnover time was short ( $\sim 7\text{--}8\text{ h}$  for a volume of  $\sim 650\text{ l}$ ). Samples were analysed for  $\delta^{18}\text{O}_{\text{seawater}}$  at the School of Environmental Sciences, University of East Anglia, by off-line equilibration with  $\text{CO}_2$  and subsequent measurement of isotope ratios using a Europa-PDZ Geo 20/20 isotope-ratio mass spectrometer, with normalisation relative to a laboratory standard, North Sea Water (accepted value of  $-0.14\text{ VPDB}$ ). The precision of replicate  $\delta^{18}\text{O}_{\text{seawater}}$  analyses is  $0.05$  ( $1\sigma$ ;  $N=30$ ) and all data are reported in per mil ( $\text{‰}$ ) deviations relative to the VPDB scale (Hut, 1987), where  $\delta^{18}\text{O}_{\text{seawater}}(\text{VPDB}) = \delta^{18}\text{O}_{\text{seawater}}(\text{SMOW}) - 0.27$ . Salinity was determined using an AutoSal 8400 Autosalinometer calibrated with International Association for Physical Sciences of the Ocean (I.A.P.S.O.) standard seawater (analytical accuracy and resolution of  $\pm 0.003$  equivalent PSU) for a subset of samples that covered the entire range of the  $\delta^{18}\text{O}_{\text{seawater}}$  variation. The temperature and  $\delta^{18}\text{O}_{\text{seawater}}$  data were collected at a higher than weekly frequency, hence average values were calculated for each growth interval.

## 2.2 Field Culturing Experiment

Specimens of the bivalve *M. edulis* were suspended  $1\text{ m}$  below a moored raft in the Menai Strait (north Wales, UK; Fig. 1) from the 8th December 2004 to the 12th December 2005. The animals were all less than  $1\text{ y}$  old when deployed, obtained from one spat cohort and initially ranged from  $2.0$  to  $2.7\text{ cm}$  in shell length. This raft is moored in the close vicinity (ca.  $500\text{ m}$ ) of the School of Ocean Sciences, Bangor University in a section of the Menai Strait where the water column is completely mixed, due to strong turbulent tidal mixing (Harvey, 1968). Animals were deployed in mesh cages and each shell was identified by a mark hand drilled on its surface. Two different, but parallel, experimental approaches were taken: 1) “short” deployment specimens were placed into cages for 16 short, well-defined and consecutive growth intervals that together covered the duration of the entire field experiment. The duration of each growth interval varied during the experiment according to expected seasonal changes in shell growth rate; 2)

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In contrast to the short specimens, “annual” deployment specimens were placed in the field for the entire duration of the experiment. To ensure that short-deployment specimens were in the same physiological condition as their annual counterparts, and to avoid the inevitable period of acclimation if animals were deployed directly from laboratory conditions, the former were taken at the start of each growth interval from a stock of animals deployed at the beginning of the experiment and kept in a separate cage.

At the end of each growth interval (which also was the beginning of the next growth interval) all short-deployment specimens from the preceding deployment and all annual specimens were removed from the raft, together with a new set of short-deployment specimens taken from the stock that were to be deployed during the next growth interval. All of these shells then were photographed and digitally imaged using the ANALYSIS software package. Between each deployment growth interval both short- and annual-deployment *M. edulis* specimens were exposed to the air for 5 to 6 h resulting in emplacement of a disturbance mark on the surface of the shells. The combination of disturbance marks and photographs was used to identify and measure all shell growth for each growth interval, as well as shell height (i.e. the distance from the umbo to the shell margin along the main axis of growth), and thus provide a time control of the new shell growth laid down throughout the field experiment by assuming shell growth rate to be constant during each growth interval.

Seawater temperature was monitored every two hours throughout the experimental deployment period using submerged temperature loggers placed in the mesh cages containing the animals (Gemini Data Loggers TinyTag – TGI 3080; accuracy of  $\pm 0.2^{\circ}\text{C}$ ). Surface seawater samples for measurement of  $\delta^{18}\text{O}_{\text{seawater}}$  were collected every two to five weeks, in the vicinity of the moored raft, using sealed salinity Winchester glass bottles. Samples were analysed for  $\delta^{18}\text{O}_{\text{seawater}}$  as described in section.

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## 2.3 Shell preparation and surface milling

### 2.3.1 Laboratory culturing experiment

The surfaces of the left hand valve of *P. maximus* shells were cleaned with a brush and any encrusting material removed using a 0.4 mm wide steel carbide burr (Minerva Dental Ltd) attached to a hand-held dental drill. The left hand valve of *M. edulis* shells were cleaned in a similar manner to the *Pecten* shells but, in addition, the outer organic periostracum was milled away with the drill until periostracum-free shell was visible in the entire sampling area. Shell powder samples subsequently were taken from the new shell growth by milling to a depth of ca. 200  $\mu\text{m}$ . Accurate milling was completed under a binocular microscope fitted with an eyepiece graticule, and depth and width of milling were controlled carefully. Each milled powder sample was taken from the main axis of shell growth: in *P. maximus* from the mid 2–3 axial ridges (ribs), and in *M. edulis* from the middle section, to avoid the increase in shell curvature that occurs away from the main growth axis (Fig. 2). Only one powder sample was milled from each individual growth interval and, particularly at the lower temperatures, the milled powder from one or more growth intervals had to be combined to provide enough shell material for analysis. Whenever the amount of sample permitted, single milled powder samples were split into separate aliquots for Mg/Ca and stable-isotope ratio determinations, otherwise only Mg/Ca ratios were measured.

Lorens and Bender (1980) have described that the stress of capture and adaptation to a new laboratory environment induced the deposition of a region of shell (termed “transition zone calcite” by those authors) with higher Mg/Ca ratios. Therefore, it is possible that the regular handling disturbance (for measurement purposes) imposed on the animals of both species during the present study may explain some of the variability of shell calcite Mg/Ca ratios that have been observed. However, Lorens and Bender (1980) only inferred the “transition zone” between new and old calcite using spot chemical compositions measured by electron microprobe, and their transition zone that exhibited higher Mg/Ca ratios occurs perpendicular to the shell surface, whereas

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sequential shell growth occurs at an angle to the shell surface. Also, no spatial scale was reported in the Lorens and Bender (1980) study making it difficult to determine the size of the region of “transition zone calcite”. Therefore, in the current study the sampling of individual growth intervals by hand-milling of powder samples between the disturbance marks on the surface of the shell, that represent the times of immersion during the experimental period, has minimized the influence of handling disturbances on measured Mg/Ca ratios.

### 2.3.2 Field culturing experiment

The left hand valve of two short-deployment *M. edulis* specimens were sampled for each growth interval, while three annual *M. edulis* specimens (A2, A6, A20) were sequentially sampled for all growth intervals. The milling of shell powder samples was as described for the laboratory culturing experiment. Whenever the amount of shell growth permitted more than one sample was collected from a single growth interval. On such occasions the new shell growth was equally divided between the number of samples collected ( $2 \leq N \leq 4$ ).

### 2.4 Shell stable-isotope and elemental ratio analyses

The shell milled powder sample preparation and analytical methodologies used in this study are as described in detail in Freitas et al. (2005; 2006). Shell and water oxygen stable-isotope data are reported in per mil (‰) deviations relative to VPDB and VSMOW, respectively. The overall analytical precision for shell  $\delta^{18}\text{O}$  measurements based on analyses of an internal laboratory standard run concurrently with all *M. edulis* and *P. maximus* samples analysed in this study is 0.08‰ (1  $\sigma$ ; N=32). Sufficient material was not available from any one growth interval to enable replicate stable-isotope analyses for an assessment of true sample precision; however, Freitas et al. (2006) used the same method as reported here and obtained a  $\delta^{18}\text{O}$  precision of 0.06‰ for five replicate measurements of the same milled powder sample obtained from one *P.*

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*maximus* specimen.

Calibration for Mg/Ca ratio determinations was performed via an established ICP-AES intensity-ratio method (de Villiers et al., 2002), using synthetic standard solutions in the range 0 to 25 mmol/mol for Mg/Ca, and most at Ca concentrations of 50 (N=304) and 60 µg/ml (N=161). The smallest milled powder samples were analysed at 30 µg/ml (N=102). Measurements were made using the Perkin Elmer Optima 3300RL ICP-AES instrument housed at the NERC ICP Facility, Royal Holloway University of London. Instrumental drift was monitored by running an intermediate (16 mmol/mol) calibration standard every 5 to 10 samples and data then were corrected accordingly. Analytical precision (expressed as relative standard deviation or RSD) was 0.5% for the laboratory cultured specimens (N=86) and 1.3% for the field cultured specimens (N=29). In the laboratory culturing experiments, sufficient material was not available from any one growth interval to enable replicate analyses for an assessment of true sample precision; in the field experiment, however, sample precision was better than 6.2% RSD for replicate measurements (N=3) of the same milled powder samples obtained from five *M. edulis* specimens. Furthermore, Freitas et al. (2006) used the same method as reported here and obtained a Mg/Ca ratio precision of 3.5% RSD for five replicate measurements of the same milled powder sample obtained from one *P. maximus* specimen. For comparison with past and future datasets, Mg/Ca ratio measurements also are reported for a set of solutions prepared by the Elderfield group at the University of Cambridge, UK (Greaves, personal communication, 2003; cf. de Villiers et al., 2002), as well as for three solutions (BAM-RS3, ECRM-752 and CMSI-1767) that have been proposed as certified reference materials (CRMs) for Mg/Ca ratio measurements in carbonates (Greaves et al., 2005) and that are subject to an ongoing international inter-laboratory calibration study (Table 2). For each CRM, approximately 50 mg of powder was dissolved in 50 g of 0.075 M HNO<sub>3</sub> (Merck Ultrapur), resulting in Ca concentrations in solution of ca. 400 µg/ml. Subsequently, 1.5 ml of each solution was centrifuged for 10 min and an aliquot then was pipetted into clean 12 ml centrifuge tubes and diluted to 10 ml to give final Ca concentrations of 50 and 30 µg/ml in order to match the sample

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and standard solutions. The linearity of the intensity-ratio calibration lines, combined with the independent assessment of the accuracy of the analytical procedure (Freitas et al., 2005, 2006), confirms the veracity of the *M. edulis* and *P. maximus* Mg/Ca ratios obtained in this study.

## 5 2.5 Statistical analyses

Two-sample *t*-tests were used to determine statistically whether significant differences existed between measured shell Mg/Ca ratios precipitated at different seawater temperatures in pairs of constant-temperature aquaria. Herein, probability levels less than 5% ( $p < 0.05$ ) are considered significantly different. Linear regressions and ANOVA analyses of shell Mg/Ca ratios and seawater temperature were performed using the software package MINITAB. Regressions were compared by testing the equality of variance in the regression residuals, since unequal variance in the regression residuals (*F*-test,  $p < 0.05$ ) indicates significantly different regressions. GLM ANOVA was used to test for differences in the slope and intercepts of the regressions. The variability in shell Mg/Ca ratios attributable to different factors was determined using fully nested ANOVA.

## 3 Results

### 3.1 Culture conditions and confirmation of shell precipitation in thermodynamic equilibrium

Seawater temperature was stable during laboratory culturing experiment one, but more variable during experiment two, especially in the lower (10°C) and mid (15°C) temperature aquaria (Fig. 3, <http://www.biogeosciences-discuss.net/5/531/2008/bgd-5-531-2008-supplement.pdf>). Nevertheless, clear temperature differences were maintained in the three different aquaria in each of the experiments (Fig. 3). Aquaria mean seawater temperatures were  $11.96 \pm 0.12^\circ\text{C}$ ,  $15.61 \pm 0.12^\circ\text{C}$

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and  $18.39 \pm 0.05^\circ\text{C}$  during experiment one, and  $10.76 \pm 0.41^\circ\text{C}$ ,  $15.54 \pm 0.25^\circ\text{C}$  and  $20.23 \pm 0.22^\circ\text{C}$  during experiment two. Variation of  $\delta^{18}\text{O}_{\text{seawater}}$  was different in the two experiments, with variable but increasing values from  $-0.10$  to  $0.10\text{‰}$  during experiment one. In experiment two  $\delta^{18}\text{O}_{\text{seawater}}$  decreased from initially high values ( $\sim 0.08\text{‰}$ ) to the lowest values observed ( $-0.33\text{‰}$ ) in the middle of the experiment and then increased to  $0.00\text{‰}$ .

During the field culturing experiment, seawater temperature exhibited a clear seasonal pattern;  $\delta^{18}\text{O}_{\text{seawater}}$  was less seasonally variable (Fig. 4a, <http://www.biogeosciences-discuss.net/5/531/2008/bgd-5-531-2008-supplement.pdf>). Seawater temperature decreased from ca.  $10.0^\circ\text{C}$  in December 2004 to a minimum temperature of ca.  $5.0^\circ\text{C}$  at the end of February, followed by a rise to ca.  $9.5^\circ\text{C}$  during mid March–late April (from d 105 to 140) and then a further rise up to a maximum temperature of ca.  $19.0^\circ\text{C}$  in early–mid July (ca. d 225). From that time to early September (ca. d 280) seawater temperature remained high at ca.  $18.0^\circ\text{C}$ , before decreasing to ca.  $9.0^\circ\text{C}$  in December 2005. During the field culturing experiment,  $\delta^{18}\text{O}_{\text{seawater}}$  varied from  $-0.48$  to  $0.03\text{‰}$ , with a mean value of  $-0.20$  ( $\pm 0.13$ ;  $N=40$ ) $\text{‰}$ , with lower values usually occurring during winter and higher values during spring and summer (Fig. 4a, <http://www.biogeosciences-discuss.net/5/531/2008/bgd-5-531-2008-supplement.pdf>).

The variation of oxygen-isotope data ( $\Delta\delta^{18}\text{O}_{\text{carbonate-seawater}} = \text{shell}\delta^{18}\text{O}$  values minus seawater  $\delta^{18}\text{O}$  values) of *M. edulis* and *P. maximus* with temperature (Fig. 5) was compared to previously derived data for the precipitation of inorganic calcite from seawater in oxygen-isotope thermodynamic equilibrium (Kim and O’Neil, 1997), but also to species-specific palaeotemperature equations obtained for *P. maximus* (Chauvaud et al., 2005) and *M. edulis* (Wanamaker et al., 2007). *P. maximus* and *M. edulis*  $\Delta\delta^{18}\text{O}_{\text{carbonate-seawater}}$  values at each temperature are similar for both species, similar for laboratory- and field-grown *M. edulis* specimens and similar to oxygen-isotope equilibrium values (Fig. 5). Thus, during both the laboratory and field culturing experiments deposition of new shell material occurred in or close to oxygen-isotope equilibrium.

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## 3.2 Shell Mg/Ca records and variability of shell calcite mg/ca ratios from laboratory cultured *M. edulis* and *P. maximus*

Measured shell Mg/Ca ratios range from 2.84 to 9.50 mmol/mol in laboratory-cultured *M. edulis* (experiments one and two) and from 8.08 to 29.92 mmol/mol in *P. maximus* (experiment two) over the experimental temperature range (Fig. 6a, <http://www.biogeosciences-discuss.net/5/531/2008/bgd-5-531-2008-supplement.pdf>). Four main features are clear from the laboratory aquaria cultured shell Mg/Ca ratio data: 1) Variability of shell Mg/Ca ratios at each temperature is very large for both species (Figs. 6a and 7). 2) Despite the high degree of variability, a significant ( $p < 0.001$  for both species), albeit weak, correlation exists between seawater temperature and shell Mg/Ca ratios in both species ( $r^2 = 0.38$  and  $0.57$  for *M. edulis* in experiments one and two, respectively;  $r^2 = 0.21$  for *P. maximus* in experiment two). 3) In experiment two, during which both species were grown at the same temperatures and in the same aquaria, shell Mg/Ca ratios are approximately three times higher in *P. maximus* than in *M. edulis* ( $t$ -test,  $p < 0.001$ , degrees of freedom  $\geq 41$  for all temperatures). 4) For *M. edulis* that was cultured in both experiment one and two there is a significant difference in the Mg/Ca ratio to temperature relationship between experiments and shell Mg/Ca ratios were higher in experiment two than in experiment one (Fig. 6a).

Irrespective of the observation that shell Mg/Ca ratios are significantly, albeit weakly, correlated with temperature in both bivalve species, the significant differences evident in the absolute shell Mg/Ca ratios of the two species, evident in the laboratory-culturing experiment, indicate a clear species-specific Mg/Ca ratio–temperature relationship for the two bivalve species investigated in this study. Furthermore, the degree of variability of shell Mg/Ca ratios at each aquaria temperature also is higher in *P. maximus* than in *M. edulis*. Unequal variance in the residuals indicates significantly different regressions of Mg/Ca ratios with temperature between *M. edulis* and *P. maximus* from laboratory-culturing experiment two ( $F$ -test,  $p < 0.05$ ). The linear fit of Mg/Ca ratios (mmol/mol) to

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seawater temperature (°C), with 95% confidence intervals, is as follows:

$$M. edulis : \text{Mg/Ca} = 1.286(\pm 0.84, ) + 0.320(\pm 0.072) * T (N = 59) \quad (1)$$

$$P. maximus : \text{Mg/Ca} = 9.886 \pm 2.96 + 0.520 \pm 0.19 * T (N = 111) \quad (2)$$

ANOVA analysis of the regressions of Mg/Ca with temperature shows that the slope of the linear regressions does not differ significantly ( $F=2.13$ ,  $p=0.146$ ), but that the intercept does ( $F=37.67$ ,  $p<0.001$ ).

*Mytilus edulis* shell Mg/Ca ratios also are statistically different between the two laboratory-culturing experiments, with higher values during experiment two (Fig. 6). The correlation between *M. edulis* shell Mg/Ca ratios and temperature also is stronger in experiment two than in experiment one ( $r^2=0.38$  and  $0.57$  in experiment one and two, respectively,  $p<0.001$  in both experiments), although this may be due solely to the smaller number of individuals analysed in experiment two compared to experiment one, i.e. the capture of a smaller degree of Mg/Ca ratio variability, as well the greater temperature range for the experiment two regression. Unequal variance in the residuals confirms significantly different regressions of Mg/Ca ratios with temperature in *M. edulis* between experiments one and two ( $F$ -test,  $p<0.05$ ). Further analysis of variance of the regressions of *M. edulis* Mg/Ca ratios and temperature shows that the slope of the regressions is not significantly different ( $F=2.50$ ,  $p=0.116$ ), but that the intercept ( $F=127.92$ ,  $p<0.001$ ) is different in the two experiments.

Evidence exists for statistically significant ( $t$ -test,  $p<0.05$ ) inter-individual shell variability of shell Mg/Ca ratios between individuals cultured within any one aquarium (Fig. 7). Maximum differences between mean shell Mg/Ca ratios from different *M. edulis* individuals cultured in the same aquarium were: 1.3 mmol/mol at 12°C (N=7), 1.9 mmol/mol at 15°C (N=6) and 2.1 mmol/mol at 18°C (N=6) in experiment one and 1.2 mmol/mol at 10°C (N=6), 3.3 mmol/mol at 15°C (N=6) and 3.0 mmol/mol at 20°C (N=6) in experiment two. For *P. maximus* maximum differences between mean Mg/Ca ratios from different individuals cultured in experiment two were: 8.9 mmol/mol at 10°C (N=8), 9.2 mmol/mol at 15°C (N=10) and 9.0 mmol/mol at 20°C (N=10).

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In addition to inter-individual shell variability, there is also a degree of intra-individual shell variability in Mg/Ca ratios within the dataset, i.e. between milled samples taken from different growth intervals (Fig. 7). For either species, the proportion of individual shells that produced Mg/Ca ratios significantly different among samples milled from the same specimen (i.e. the difference between any two Mg/Ca measurements was larger than twice the analytical error) was similar at each temperature. However, *P. maximus* showed a higher frequency of milled samples with different Mg/Ca ratios within an individual shell (>97% in all aquaria) than did *M. edulis*, (68% < experiment one < 73%, and 72% < experiment two < 83%).

### 3.3 Shell Mg/Ca Records and variability of shell calcite Mg/Ca ratios from field cultured *M. edulis*

For the field-cultured *M. edulis*, measured shell Mg/Ca ratios range from 2.96 to 9.16 mmol/mol in the short-deployment specimens; from 2.86 to 8.34 mmol/mol in the A2 specimen; from 2.78 to 5.97 mmol/mol in the A6 specimen and from 2.75 to 6.11 mmol/mol in the A20 specimen (Figs. 4b and 6, <http://www.biogeosciences-discuss.net/5/531/2008/bgd-5-531-2008-supplement.pdf>). During the field-culturing experiment, shell Mg/Ca ratios from short- and annual-deployment *M. edulis* specimens showed a clear seasonal pattern (Fig. 4b). Shell Mg/Ca ratios were low at ca. 4 mmol/mol during the winter and spring months up to May, and then increased to maxima higher than 7 mmol/mol during June–July. From that time to October shell Mg/Ca ratios remained high at ca. 7 mmol/mol, and then decreased to ca. 3.5 mmol/mol in December 2005 (Fig. 4b).

In field-cultured *M. edulis*, a significant ( $p < 0.001$  for all specimens) correlation exists between seawater temperature and shell Mg/Ca ratios (Fig. 6b):  $r^2 = 0.54$  for the short-deployment specimens ( $N = 62$ );  $r^2 = 0.77$  for the A2 specimen ( $N = 28$ );  $r^2 = 0.72$  for the A6 specimen ( $N = 34$ ) and  $r^2 = 0.81$  for the A20 specimen ( $N = 30$ ). However, the correlation between shell Mg/Ca ratios and temperature is weaker ( $r^2 = 0.50$ ,  $p < 0.001$ ) when all the data from all the specimens are pooled together. Furthermore, variance

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of the residuals was only equal for the regressions of Mg/Ca ratios with temperature for *M. edulis* specimens between short-deployment and A2 specimens and between A6 and A20 specimens ( $F$ -test,  $p>0.05$ ). In all other specimens, unequal variance of residuals indicates significantly different regressions of Mg/Ca ratios with temperature ( $F$ -test,  $p<0.05$ ). ANOVA analysis of the regressions of Mg/Ca with temperature between short-deployment and A2 specimens, and between A20 and A6 specimens, shows that the slope of the linear regressions does not differ significantly ( $F=2.70$ ,  $p=0.104$  and  $F=1.40$ ,  $p=0.242$ , respectively), but that the intercept does ( $F=126.11$ ,  $p<0.001$  and  $F=196.31$ ,  $p<0.001$ , respectively). Evidence thus exists for significant inter-individual variability of shell Mg/Ca ratios and its relationship with temperature from *M. edulis* specimens grown under the same field-culturing conditions. Maximum shell Mg/Ca ratios, in particular, are markedly different between individual specimens and range from 5.97 to 9.16 mmol/mol.

For the same range of temperature, shell Mg/Ca ratios of *M. edulis* grown in the laboratory- and field-culturing experiments showed a similar range (Fig. 6). However, the correlation between Mg/Ca ratios and temperature was stronger in field-cultured ( $0.54<r^2<0.81$ ) than in laboratory-cultured *M. edulis* specimens ( $0.38<r^2<0.57$ ). Furthermore, ANOVA analysis of the regressions of Mg/Ca with temperature between laboratory-cultured and field-cultured *M. edulis* specimens shows that the slope of the linear regressions does not differ significantly ( $F=0.70$ ,  $p=0.799$ ), but that the intercept does ( $F=224.68$ ,  $p<0.001$ ).

## 4 Discussion

### 4.1 Inter-species, inter-individual and intra-individual variability in shell Mg/Ca ratios

In addition to the weak, but significant, relationships with seawater temperature, the shell Mg/Ca ratio data obtained in this study also clearly show a large degree of variability in absolute shell Mg/Ca ratios in both *M. edulis* and *P. maximus* species (Figs. 4,

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6 and 7). Like with other bivalve geochemical and physical proxies (for review see e.g. Richardson, 2001) variability of shell Mg/Ca ratios occurs at different levels, requiring consideration of differences between the two bivalve species cultured (inter-species level), between shells of different individuals grown simultaneously in the same aquarium or in the same cages and under the same laboratory- and field-culturing conditions (inter-individual shell level) and within individual shells, i.e. between milled samples taken from one individual shell that correspond to different growth intervals during the experimental period (intra-individual shell level).

Differences in shell Mg/Ca ratios of the same species have been observed in previous field-based studies at levels of both inter- and intra-individual shell variability (Rosenberg and Hughes, 1991; Klein et al., 1996; Vander Putten et al., 2000; Freitas et al., 2005; Lorrain et al., 2005; Freitas et al., 2006). For example, Klein et al. (1996) presented data from two field-collected shells (British Columbia, Canada) of the mussel *M. trossulus* which clearly show large Mg/Ca ratio differences at inter- (up to 2.5 mmol/mol) and intra- (up to 1.5 mmol/mol) individual shell levels, in addition to a temperature relationship ( $r^2=0.74$ ,  $p<0.001$ ) over a range from 5.5 to 22.7°C. By comparison, Vander Putten et al. (2000) reported inter-individual differences in Mg/Ca ratios between four *M. edulis* field-grown shells (Schelde Estuary, Netherlands) as high as ~7 mmol/mol. Similarly, Lorrain et al. (2005) presented data from four *P. maximus* specimens collected from the Bay of Brest, France, where differences of up to 6 mmol/mol in Mg/Ca ratios were observed between individual specimens for shell samples that corresponded to the same time of calcification. Most recently, in three *P. maximus* specimens grown in a field-based experiment, and for a similar temperature range to that used in the present laboratory culturing study (10 to 20°C), differences were observed in Mg/Ca ratios of up to 7.5 mmol/mol between shells (Freitas et al., 2006). Specifically, for  $\delta^{18}\text{O}$ -derived calcification temperatures of  $10\pm0.5^\circ\text{C}$  (N=8) and  $15\pm0.5^\circ\text{C}$  (N=6) Mg/Ca ratios varied by up to 4.06 and 5.61 mmol/mol, respectively (Freitas et al., 2006). The inter- and intra-individual variability of Mg/Ca ratios in shell calcite of *M. edulis* and *P. maximus* grown in field-based and laboratory culturing stud-

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ies thus are of similar magnitude.

Significant differences in absolute shell Mg/Ca ratios can be observed between the two cultured bivalve species (Fig. 6); *P. maximus* shell Mg/Ca ratios being approximately three times higher than those in *M. edulis*. Large variations in the Mg content of biogenic calcite from different species has been observed previously in bivalves (Lorens and Bender, 1980; Klein et al., 1996; Vander Putten et al., 2000; Lorrain et al., 2005). The Mg/Ca ratio data obtained in this study for laboratory cultured *M. edulis* and *P. maximus* have been compared to previously published data for other marine bivalve species investigated in field-based studies (Fig. 8). On the whole, a large degree of overlap can be observed between the Mg/Ca ratio data derived from the laboratory- and field-cultured *M. edulis* and *P. maximus* specimens. Nevertheless, laboratory- and field-cultured *M. edulis* show lower shell Mg/Ca ratios than data reported from field experiments for *M. edulis* by Vander Putten et al. (2000), although the latter data were obtained by laser ablation ICP-MS and there is the potential for calibration issues between datasets. The Mg/Ca ratios for *M. edulis* cultured in this study are, however, similar to Mg/Ca ratios reported for *M. trossulus* (Klein et al., 1996), a close relative of *M. edulis*. Shell Mg/Ca ratios in the *P. maximus* animals cultured in this study are similar to Mg/Ca ratios reported for specimens of the same species grown or collected in field studies (Lorrain et al., 2005; Freitas et al., 2006), but extend to higher values and also show a larger variability than in specimens grown at a field location adjacent to the present aquarium based study (Freitas et al., 2006). This latter observation suggests that the influence of any non-temperature control (i.e. a physiological control) on *P. maximus* shell Mg/Ca ratios may well be stronger under laboratory culture conditions than in field-based situations that more closely mimic the conditions best suited for optimal growth of natural populations.

#### 4.2 Imprecise temperature control on shell Mg/Ca ratios

Despite the observation that the shell oxygen-isotope composition was deposited in or near to oxygen-isotope thermodynamic equilibrium (Fig. 5), one obvious feature of

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the measured shell Mg/Ca ratios obtained from specimens of *M. edulis* and *P. maximus* cultured in the constant-temperature aquaria in this study is that there is only a weak dependence on temperature (Fig. 6a and Table 3). Nevertheless, in *M. edulis* specimens cultured in the field, shell Mg/Ca ratios were significantly correlated with temperature ( $0.54 < r^2 < 0.81$ ). However, the inter-individual variability of Mg/Ca ratios is large (Fig. 6b) and results in a weaker correlation ( $r^2 = 0.50$ ,  $p < 0.001$ ) when data from all field cultured specimens are pooled together. Furthermore, linear regressions of Mg/Ca ratios with temperature are different: between laboratory cultured *P. maximus* and *M. edulis*, between *M. edulis* specimens grown in the two laboratory culturing experiments, between laboratory- and field-cultured *M. edulis* specimens, and between individual field-cultured *M. edulis* specimens. Therefore, the establishment of even a species-specific valid regression between Mg/Ca ratios and temperature has not been possible for the two species studied.

The temperature dependent incorporation of Mg into inorganic calcite experimentally precipitated from seawater (Chilingar, 1962; Katz, 1973; Burton and Walter, 1987; Mucci, 1987; Oomori et al., 1987) and in other biogenic calcites (e.g. Dwyer et al., 1995; Nurnberg et al., 1996; Stoll et al., 2001) has been well-documented. Clearly, as has been observed previously in some field studies, the weak temperature dependence of shell Mg/Ca ratios in the two marine bivalve species that were investigated in this study is a feature specific to the incorporation of Mg into the calcite shells of these organisms and must therefore relate to their specific biomineralization processes, including any secondary physiological influences.

The suggestion of additional physiological controls on Mg/Ca ratios in bivalve calcite (i.e. metabolic or kinetic controls) is supported further by the significant difference in the absolute shell Mg/Ca ratios in *M. edulis* grown at 15°C in two aquaria in experiments one and two of ~1.1 mmol/mol ( $t$ -test,  $p = 0.004$ ,  $DF = 24$ ), but also by the large inter- and intra-individual variability of Mg/Ca ratios observed in both the laboratory- and field-cultured specimens (Fig. 6). These observations clearly indicate that specimens from the same species cultured at different times at the same seawater temper-

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ature can have different shell Mg/Ca ratios. It is therefore important to recognise that other non-thermodynamic factors in the two experiments must also have influenced shell Mg/Ca ratios. Furthermore, it is not possible to discount the possibility that the *M. edulis* animals cultured in experiment two were better conditioned for the laboratory environment than those in experiment one, due to their longer acclimation in the experimental aquaria prior to commencement of the experimental period. Consequently, the outcomes of this laboratory culturing experiment must be compared to other laboratory culturing and field-based studies in order to obtain the most robust interpretations.

Given the experimental design in this study, only factors that were entirely independent of seawater temperature can be discussed as additional potential controls on shell Mg/Ca ratios. This consideration thus prohibits a detailed discussion of the influence of shell growth rate on shell Mg/Ca ratios, since growth rates co-vary significantly with temperature in both the laboratory- (for *P. maximus*,  $r^2=0.62$ ,  $p<0.001$ ; for *M. edulis*,  $r^2=0.23$ ,  $p=0.001$  and  $r^2=0.15$ ,  $p=0.032$  in experiment one and two, respectively) and field-culturing experiments (for *M. edulis*,  $0.26<r^2<0.43$ ,  $p<0.002$ ). Nevertheless, as reported by Lorens and Bender (1980) for laboratory-cultured *M. edulis* specimens, shell Mg/Ca ratios also were only weakly correlated to shell growth rates in both the laboratory- and field-culturing experiments (Table 3).

A metabolic control, i.e. the physiological exclusion of Mg from its shell-forming fluid (the extra-pallial fluid or EPF), on calcite Mg content has been proposed previously for *M. edulis* (Lorens and Bender, 1977, 1980). Metabolic control also was suggested as a possible way of explaining an observed seasonal breakdown in the relationship between Mg/Ca and temperature reported for *M. edulis* (Vander Putten et al., 2000). An apparent ontogenetic control of Mg/Ca ratios has been described in the fan mussel *Pinna nobilis*, although a temperature control on shell Mg/Ca ratios also was present in this species (Freitas et al., 2005). For *P. maximus*, recent field-based studies have shown the absence of a significant correlation between shell Mg/Ca ratios and seawater temperature (Lorrain et al., 2005) or a strong seasonal variation in the strength of the correlation between shell Mg/Ca ratios and seawater temperatures, again suggest-

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ing that other factors must influence Mg/Ca ratios in *P. maximus* shell calcite (Freitas et al., 2006).

Seawater salinity is a truly independent variable in the laboratory culture experiment, but not in the field-culturing experiment where it co-varies with seawater temperature ( $r^2=0.50$ ,  $p<0.001$ ). Any differences in seawater salinity between the two laboratory-culturing experiments thus could influence the amount of magnesium available for incorporation, assuming that shell Mg/Ca ratios are not solely related to seawater Mg/Ca ratios. Indeed, Lorens and Bender (1980) have shown that shell Mg/Ca ratios increase with increasing solution Mg concentrations, albeit at much higher concentrations than would be expected from natural changes in seawater salinity. By comparison, an earlier study by Dodd (1965) observed the opposite trend of increasing Mg concentrations in *M. edulis* shell calcite with decreasing salinity. In addition, salinity has been reported to significantly influence the Mg/Ca ratios of foraminifera calcite (Lea et al., 1999). In *M. edulis*, salinity was not significantly correlated with shell Mg/Ca ratios in the two laboratory experiments in this study ( $p>0.05$ ), with only a weak correlation for *P. maximus* ( $r^2=0.21$ ,  $p<0.001$ ). The strength of this correlation between shell Mg/Ca ratios and salinity is, however, of comparable magnitude to that observed between temperature and shell Mg/Ca ratios ( $r^2=0.21$ ,  $p<0.001$ ). Nevertheless, temperature and salinity together ( $r^2=0.37$ ,  $p<0.001$ ) still do not explain much more of the observed shell Mg/Ca variability in *P. maximus* than just temperature alone.

#### 4.3 Are Mg/Ca ratios in bivalve calcite an unreliable palaeotemperature proxy?

Bivalves, like other calcifying organisms, are capable of regulating, or at least influencing to variable extents, the Mg content of their calcium carbonate skeletons (Dodd, 1965; Lorens and Bender, 1977; Neri et al., 1979; Onuma et al., 1979; Lorens and Bender, 1980; Rosenberg and Hughes, 1991; Rosenberg et al., 2001). This phenomenon can be expressed by temperature-dependent partition coefficients ( $D_{Mg}$ ) between the solid mineral phases and ambient seawater medium ( $D_{Mg}=Mg/Ca_{\text{calcite}}/Mg/Ca_{\text{seawater}}$ ). The differences in the Mg content of calcite secreted by different taxa, as well as dif-

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ferences in Mg/Ca ratios between and within individuals of a single species, suggest a strong physiological control of the incorporation of Mg into biogenic calcites. Examples of such physiological effects that may influence, either directly or indirectly, the Mg content of bivalve shell calcite are: variable chemical composition of the precipitating fluid, i.e. the EPF, resulting from biological control on differential transport of ions into and out of the EPF; variable calcification rates; the transport and diffusion conditions of the local precipitation microenvironment (Wasylenki et al., 2005); and differences in crystal growth orientation and morphology (Mucci and Morse, 1983; Reeder and Grams, 1987; Debeney et al., 2000; Erez, 2003).

Small-scale heterogeneous distribution of Mg may represent a particularly relevant source of error in the use of bivalve calcite Mg/Ca ratios as a palaeotemperature proxy. Lorens and Bender (1980) have described significant small-scale variability of Mg/Ca ratios, from <5 to 40 mmol/mol over scales of 100's  $\mu\text{m}$ , in the very first new shell growth from *M. edulis* cultured in natural seawater under controlled conditions at temperatures between 22 and 24°C. Small scale variations in Mg concentrations in *M. edulis* calcite have been shown to derive from Mg being concentrated along the margins of calcite prisms, especially along the terminations of the crystals, with the alignment of adjacent crystals then producing compositional growth bands within the shell (Rosenberg et al., 2001). The latter observation lead to the suggestion that in *M. edulis* Mg and also sulphur in the shell could control rates of shell crystal elongation, shell curvature along different axes and ultimately the Mg distribution throughout the shell (Rosenberg et al., 2001).

The use of Mg/Ca ratios from bivalve calcite shells as a reliable and accurate temperature proxy thus remains unlikely at present, at least in the species studied to date. The now well-documented variation of Mg/Ca ratios in bivalve calcite at species-specific, inter- and intra-individual shell levels prevents the establishment of valid Mg/Ca ratio–temperature relationships, even for individual species. Furthermore, there exists support for a strong metabolic control of Mg/Ca ratios in bivalve shells (Lorens and Bender, 1977, 1980; Rosenberg and Hughes, 1991; Vander Putten et al., 2000; Rosenberg et

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al., 2001), although the mechanisms by which such a control acts are still not fully clear, as well as for extensive small-scale heterogeneity in shell Mg contents (Lorens and Bender, 1980; Rosenberg et al., 2001). Future research should address these issues in greater detail, if ever this geochemical proxy is to be used as a reliable and accurate temperature proxy in bivalve calcite.

## 5 Conclusions

In laboratory- and field-culturing experiments only a weak dependence on temperature, as well as a large degree of variability, has been observed for shell Mg/Ca ratios in calcite sampled from two marine bivalve species, *M. edulis* and *P. maximus*. Such variability is significant at the species, inter- and intra- individual shell levels, and most likely reflects the influence of additional secondary physiological factors influencing shell biomineralisation and Mg content. Shell Mg/Ca ratios were different between *M. edulis* and *P. maximus*, being three to five times greater in the latter species. The variability of shell Mg/Ca ratios for laboratory-and field-cultured *M. edulis* in the present study was similar to the variability observed in previous field-grown specimens. Laboratory cultured *P. maximus* specimens, however, showed approximately twice the variability of shell Mg/Ca ratios than has been reported previously for field-grown specimens. In the two species, shell Mg/Ca ratios were not found to be controlled by shell growth rate or salinity. The Mg/Ca ratio data obtained in the present laboratory and field culturing of *M. edulis* and *P. maximus*, together with supporting evidence from previous field studies, clearly suggests that bivalve Mg/Ca ratios do not yet appear to be a reliable and precise temperature proxy. Strong physiological controls and extensive small-scale heterogeneity in shell Mg content may even prevent unique Mg/Ca ratio to temperature relationships for individual species being defined. Unless the physiological controls on Mg incorporation can be understood in more detail and subsequently compensated for, the use of this geochemical proxy as a reliable and accurate temperature proxy remains unlikely, at least in the bivalve species studied to date.

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**Table 1.** Start dates of the two laboratory culturing experiments and duration of growth intervals (days) in each aquarium for which new shell growth was evident.

Species	Aquarium Interval		Experiment 1																
			12°C						15°C						18°C				
			1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5
<i>M. edulis</i>	Start Date	2004	23/02						25/02						01/03				
	Duration	Days	7	7	7	7	7	9	7	7	7	7	7	6	7	7	7	7	7
Species	Aquarium Interval		Experiment 2																
			12°C						15°C						18°C				
			1	2	3	4	5	6	1	2	3	4M	4P		1	2	3M	3P	
<i>M. edulis</i> <i>P. maximus</i>	Start Date	2004	07/05						06/05						17/05				
	Duration	Days	7	7	7	7	7	7	7	7	7	7	12		7	7	10	13	

In experiment two the last growth interval was of different duration for the two species grown at 15 and 20°C and the suffixes M and P indicate the last growth interval for *M. edulis* and *P. maximus*, respectively.

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**Table 2.** Comparison of expected (Greaves et al., 2005) with measured Mg/Ca ratios for three certified reference material (CRMs) solutions.

CRM solution	Expected	Inter-laboratory	This study	Difference
BAM-RS3	0.80	0.80±0.01 (N=6)	0.78±0.12 (N=9)	−3.0
ECRM-752	3.90	3.50±0.04 (N=6)	3.82±0.07 (N=13)	−2.1
CMSI-1767	6.10	5.58±0.09 (N=6)	5.76±0.07 (N=11)	−5.7

The values in the third column are those returned from Bangor University as part of an ongoing international inter-laboratory comparison exercise, with the replicates representing six separate dissolutions of each CRM (1 ml of each solution was centrifuged for 10 min at ~6000 rpm and ca. 0.75 ml from each solution then diluted to final solution Ca concentrations of 60 µg/ml). By comparison, the replicates reported for this study in the fourth column are repeated measurements of a single dissolution completed for each CRM and diluted to Ca concentrations of 50 or 30 µg/ml. All measurements were made on the same Perkin Elmer Optima 3300RL ICP-AES instrument.

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**Table 3.** Summary of correlations between Mg/Ca ratios and temperature, shell growth rate (SGR –  $\mu\text{m/d}$ ) and salinity for all laboratory- (experiment one and two) and field-culturing experiments.

Experiment	Temperature		SGR		Salinity	
	$r^2$	$p$	$r^2$	$p$	$r^2$	$p$
Laboratory						
<i>M. edulis</i> 1	0.38	<0.001	0.19	0.006	–	>0.05
2	0.57	<0.001	0.33	<0.001	–	>0.05
1+2	0.37	<0.001	0.23	<0.001	–	>0.05
<i>P. maximus</i> 2	0.21	<0.001	0.09	0.002	0.21	<0.001
Field						
<i>M. edulis</i>	0.50	<0.001	0.22 to 0.41	<0.004	–	–

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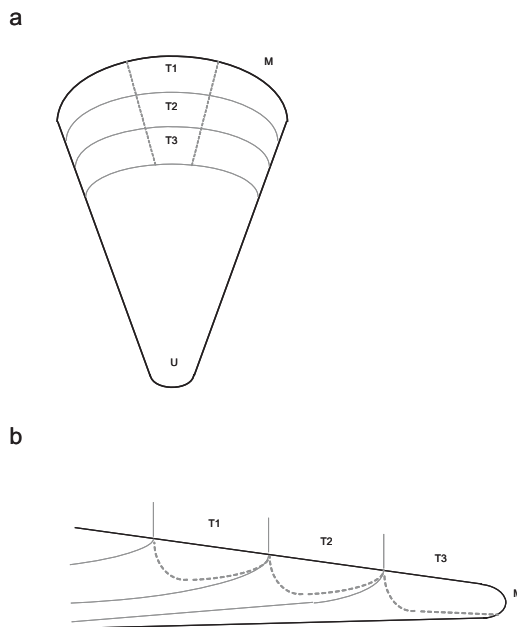
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**Fig. 1.** Location of the field deployment site, Menai Strait, Wales, UK.

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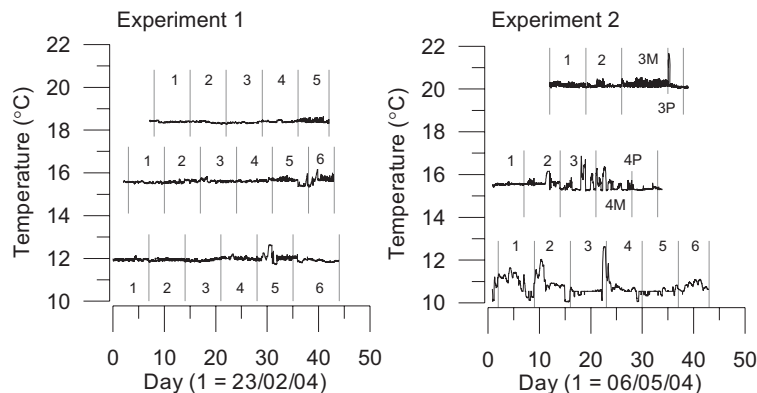


**Fig. 2.** Schematic representation of a shell to indicate the sampling approach for *M. edulis* and *P. maximus* shells. **(a)** View of the outer shell surface from above and **(b)** Longitudinal section of the shell. M is the shell margin; U is the shell umbo; grey lines define the boundaries between individual growth intervals identified by T1, T2 and T3. Samples of shell calcite were collected for each growth interval along the main axis of growth (delimited by dashed lines in (a), avoiding areas of excessive shell curvature, and up to a depth of ca. 200  $\mu\text{m}$  in the areas delimited by the dotted lines in (b)).

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**Fig. 3.** Variation of seawater temperature measured every 15 min in all the laboratory aquaria during experiments one and two. Vertical lines define limits of growth intervals in each tank. In experiment two, the last growth interval was of different duration for the two species, and the suffixes M and P indicate the last growth interval for *M. edulis* and *P. maximus*, respectively.

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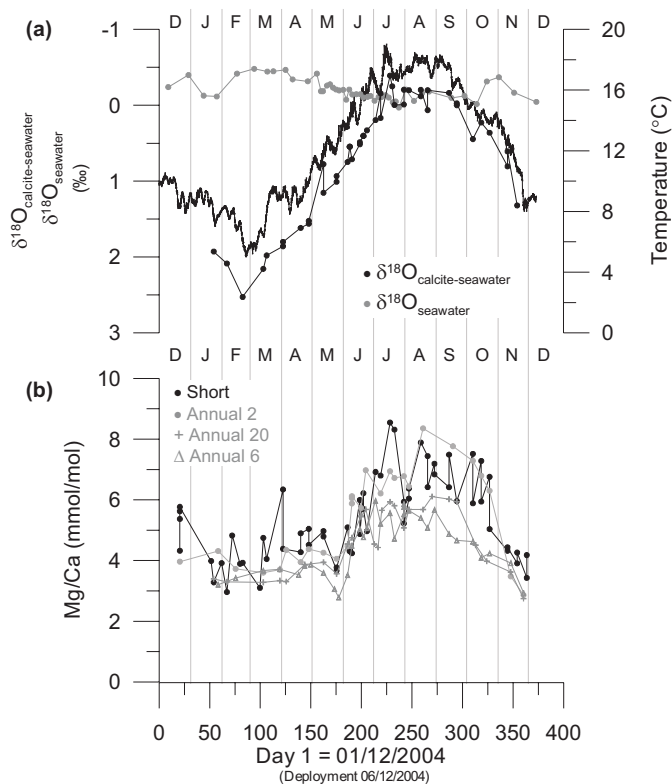
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**Fig. 4.** (a) Variation of seawater temperature,  $\delta^{18}\text{O}_{\text{seawater}}$  and  $\delta^{18}\text{O}_{\text{calcite-seawater}}$  (for *M. edulis* short-deployment specimens only) during the field-culturing experiment. Note that the  $\delta^{18}\text{O}$  scale has been inverted to correspond with seawater temperatures increasing upwards. (b) Variation of shell Mg/Ca ratios of *M. edulis* (annual- and short-deployment specimens) during the field-culturing experiment. Vertical lines define limits of calendar months and letters indicate calendar month.

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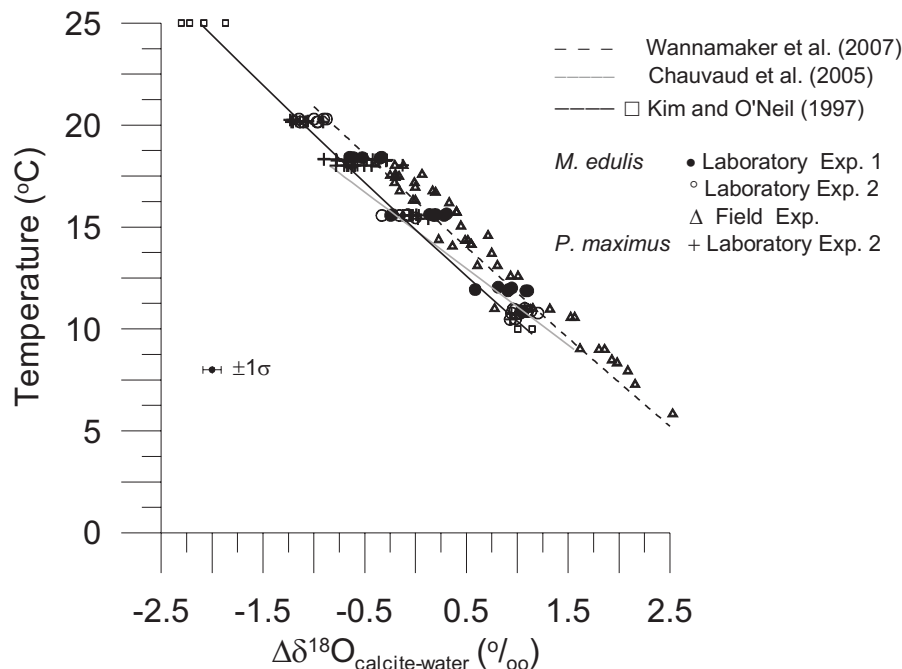
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**Fig. 5.** Seawater temperatures plotted against mean  $\Delta\delta^{18}\text{O}$  values ( $\delta^{18}\text{O}_{\text{carbonate}} - \delta^{18}\text{O}_{\text{seawater}}$ , on the VPDB and VSMOW scale, respectively) for laboratory-cultured *M. edulis* (• – experiment one and ◦ – experiment two) and *P. maximus* (+ experiment two only), and field-cultured *M. edulis* (Δ). Plotted also are the data (◻ – solid black line) for inorganic calcite deposited from seawater in oxygen-isotope thermodynamic equilibrium from Kim and O'Neil (1997), but also species-specific palaeotemperature equations obtained for *P. maximus* (solid grey line) by Chauvaud et al. (2005) and *M. edulis* (dashed black line) by Wannamaker et al. (2007). Due to the use of different acid fractionation factors between the present study and Kim and O'Neil (1997), 0.25 was subtracted from their original  $\delta^{18}\text{O}_{\text{carbonate}}$  values. For comparison, twice the analytical error for  $\delta^{18}\text{O}_{\text{carbonate}} - \delta^{18}\text{O}_{\text{seawater}}$  ( $\pm 0.09\text{‰}$ ) also is shown.

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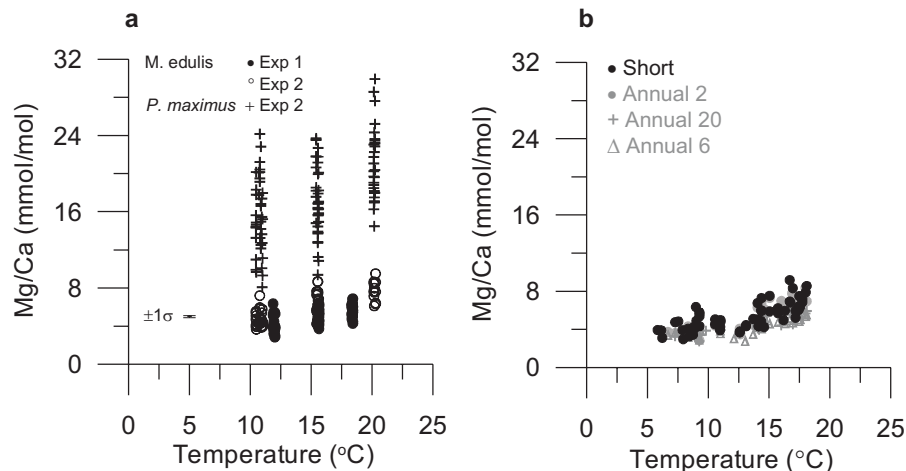
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## Variability of Mg/Ca ratios in bivalve shell calcite

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**Fig. 6.** Shell Mg/Ca ratios plotted against seawater temperature from: **(a)** laboratory-cultured *M. edulis* (● – experiment one and ○ – experiment two) and *P. maximus* (+ experiment two only); **(b)** field-cultured *M. edulis* (● – short-deployment specimens; annual-deployment specimens: ● – A2, Δ – A6 and + A20). Each point represents a paired seawater temperature value and Mg/Ca ratio obtained for individual growth intervals. For comparison, twice the analytical error ( $\pm 0.10$  mmol/mol) also is shown.

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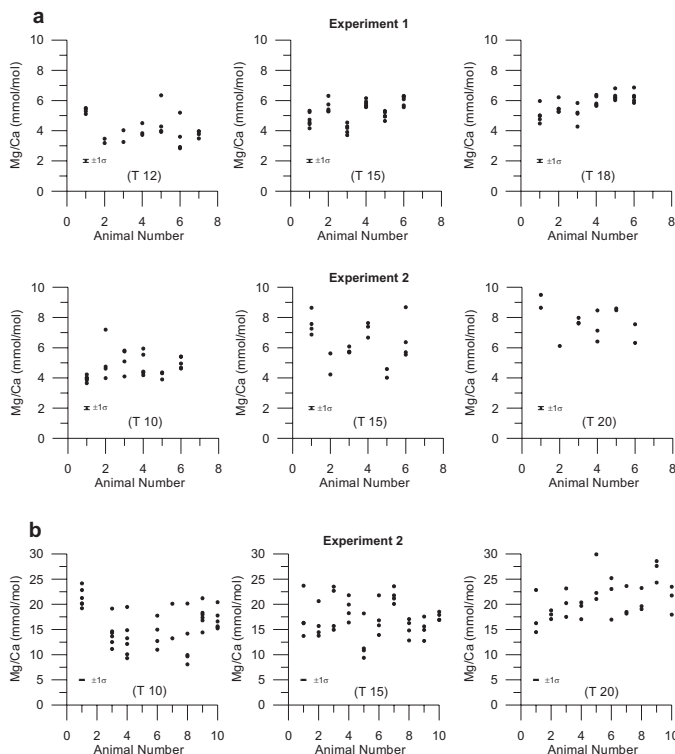
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# Variability of Mg/Ca ratios in bivalve shell calcite

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**Fig. 7.** Shell Mg/Ca ratios plotted against animal number for each aquarium (temperature in brackets) in order to illustrate inter- and intra-individual shell variability of Mg/Ca ratios in **(a)** *M. edulis* and **(b)** *P. maximus*. For each animal, individual data points correspond to Mg/Ca ratios of new shell growth deposited in the experiment during different growth intervals. For comparison, twice the analytical error ( $\pm 0.10$  mmol/mol) also is shown.

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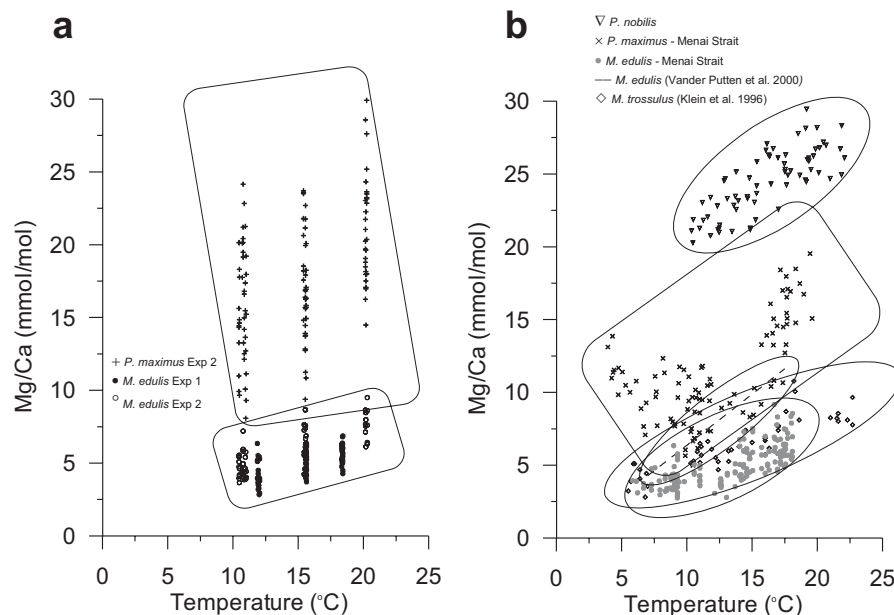
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# Variability of Mg/Ca ratios in bivalve shell calcite

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**Fig. 8.** Comparison of bivalve calcite shell Mg/Ca ratios, plotted against temperature, from: **(a)** laboratory aquaria culturing completed in this study for *Pecten maximus*<sup>1</sup> and *Mytilus edulis*<sup>1</sup>; and **(b)** field culturing completed in this study for *Mytilus edulis*<sup>1</sup> and other field-based studies, for the species: *Mytilus edulis* (Vander Putten et al., 2000)<sup>1</sup>, *Pecten maximus* (Freitas et al., 2006)<sup>2</sup>, *Mytilus trossulus* (Klein et al., 1996)<sup>1</sup> and *Pinna nobilis* (Freitas et al., 2005)<sup>2</sup>. [<sup>1</sup> denotes temperature is a measured seawater temperature; <sup>2</sup> denotes temperature is a  $\delta^{18}\text{O}_{\text{calcite}}$ -derived calcification temperature].

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